MUSCLE TISSUE CHANGES IN EXPERIMENTAL FROSTBITE*

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The necrosis of tissues subsequent to local exposure to cold, experimentally, may be caused by the direct physico-thermal action of the low temperature on tissue cells or may be produced by neurovascular changes of vasoconstriction, thrombosis and anoxia.

Investigations on muscle tissue were performed with the object of correlating the observed changes with one of these mechanisms. Muscle tissue was chosen because of the high sensitivity to cold.[3] It is possible to produce muscle necrosis without causing damage to the overlaying skin, which could lead to secondary infection.[14] Further, the morphologic changes might be easily detected in both the muscle cells and in the vascular system of the musculature.

MATERIAL AND METHODS

Males of the Royal Victoria Hospital strain of hooded rats, 180–220 Gm. in weight, and male albino rabbits, 2250–2750 Gm. in weight, were used. Both hind legs were depilated before experiments. The animals were anesthetized with intraperitoneal injections of sodium pentobarbital, (Abbott). One of the depilated legs was immersed in the fluid containing ethylene glycol, ethyl alcohol and distilled water, and cooled by solid carbon dioxide to the desired temperature according to the procedure used by Fuhrman and Crismon.[8] One leg of each animal was frozen solid by exposure to the medium for four minutes at −29°C to −30°C for rats, and four minutes at −35°C to −37°C for rabbits. This produced a standard injury to the tissues. The legs were allowed to thaw in air at room temperature.

The animals were sacrificed in groups of two at two, four, six, eight, 12, 18, 24, 36, 48 and 76 hours after exposure. Immediately after killing, parts of skin, subcutaneous tissue and the tibialis anticus muscle were removed and placed in Carnoy fixative, subsequently embedded in paraffin and stained with hematoxylin and eosin and van Gieson stain.

GROSS FINDINGS

On thawing, the tissues swell rapidly and the skin becomes intensely red. The swelling remains constant up to six to 12 hours, the skin coloration varies to red bluish during this time. Blistering and exudation of the skin can be observed. The edema fluid changes from a viscous to a liquid consistency during the course of 48 hours. In some animals it was slightly bloody eight to 12 hours after freezing. During the first hours the muscles were of dull red coloration and contained easily coagulable edema fluid, but in later stages the color appeared greyish-red without signs of gross necrosis.

MICROSCOPIC FINDINGS

Two hours after exposure (Fig. 1A) the histologic structure of the exposed muscle appeared completely disrupted. The muscle fibers were expanded beyond the normal structural boundaries and the interstitial spaces were enlarged by edema

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fluid. Many fibers presented a wavy appearance. Muscle cells were variously fragmented and segmented into transverse disks and fenestrated by irregularly-shaped vacuoles. The muscle fibers in certain areas were shrunken, producing variable outlines. Some showed complete absence of protoplasm. Such morphologic changes produced debris of nuclei and eosinophilic granules of crumbling sarcoplasm could be seen. The fibrous network of endomysium was enlarged, containing some swollen fibroblast and present macrophages showed deeply basophilic protoplasm.

The capillaries and larger blood vessels were dilated and filled densely with erythrocytes but no

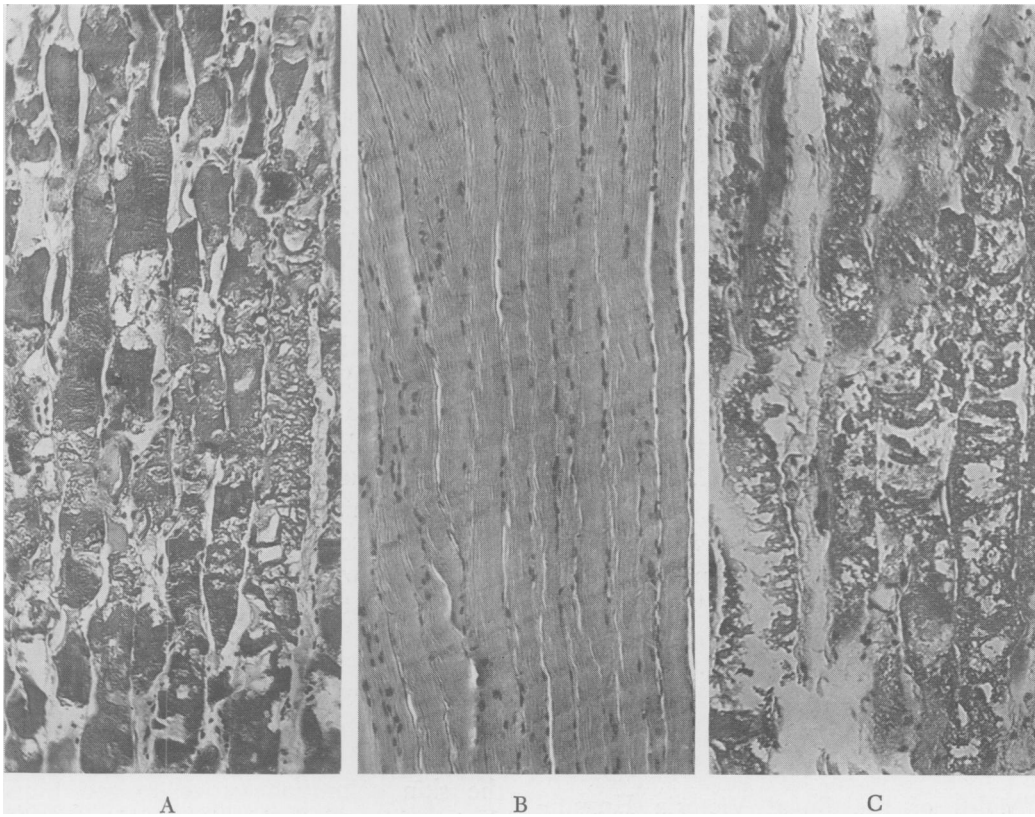

FIG. 1.—(A) Degenerative changes of the muscle cells two hours after exposure to severe cold. Adult rat. H. and E. x 120. (B) Control section taken from unexposed muscle of the same animal as in Fig. 1A, H. and E. x 100. (C) Degeneration and disintegration of the muscle fibers four hours after cold injury. Adult rat. H. and E. x 125.

staining abberations. The sarcolemma could be seen in the injured muscle fibers as a thin membrane bridging the absent portion of the sarcoplasm, or as a ruptured membrane attached to the degenerated muscle cell. The myofibrillar structure was almost completely lost; the sarcoplasm showed homogeneity or gave an appearance of disintegration in differently shaped pieces. The nuclei were absent in some areas of injured fibers; in others they were pyknotic or fragmented.

The interstitial spaces were enlarged and filled with eosinophilic edema which appeared homogenous or granular. In the spaces close to the disintegrating muscle cells, parts of broken sarcolemma, thrombi could be observed. The minute blood vessels had enlarged endothelial cells with more basophilic nuclei.

The corresponding muscle of the unexposed leg of the same animal (Fig. 1B) did not show any abnormalities.

Four hours after exposure (Fig. 1C), there was evidence of more extensive sarcolysis of muscle fibers. Vacuolisation and fenestration was more extensive and a larger number of fibers lost parts of their sarcoplasm, resulting in a honey-combed eosinophilic structure. More muscle cells could be observed to have broken or pyknotic nuclei. The interstitial spaces were filled with edema, con-

taining more debris of nuclei, sarcoplasm and sarcolemma. Fibroblasts appeared to be more swollen. No thrombi could be observed in the dilated, erythrocyte filled blood vessels.

The maximum changes in the morphology of muscle tissue were seen 6 hours after exposure. The disintegration of muscle tissue was so extensive as to produce a picture of complete disruption.

The capillaries and larger blood vessels were dilated and well filled with blood elements, but no thrombosis could be seen. However, the larger blood vessels showed edema fluid infiltration into the wall.

Sections from animals taken 8 hours after exposure showed identical sarcolytic destruction of cellular structure and failure to take staining. The necrotic process in the musculature seemed to be completed at this time and the stage of repair had begun. This is shown by the infiltration of polymorphonuclear cells and macrophages into the interstitial spaces and amorphous, necrotic muscle fibers. Blood vessels were distended but free of thrombi.

Twelve to 18 hours after injury there was no increase in muscle cell necrosis. Polymorphonuclear cells were abundant, some eosinophilic leukocytes and a few mononuclear cells as well as macrophages were seen in interstitial spaces. The leukocytes and mononuclear cells surrounding and indenting the necrobiotic muscle cells indicated phagocytosis. The amount of interstitial edema decreased and contained much debris, probably due to the decay of migrating cells. The blood vessels failed to contain thrombi.

Sections taken from animals 18 to 24 hours after exposure showed no further necrosis of muscle fibers. More polymorphonuclear and mononuclear cells were visible in interstitial spaces. These were grouped around necrotic muscle cells predominantly in the areas in proximity to intact muscle fibers. The proliferation of connective tissue cells, replacing necrotic muscle fibers, began around the blood vessels. No thrombi could be observed.

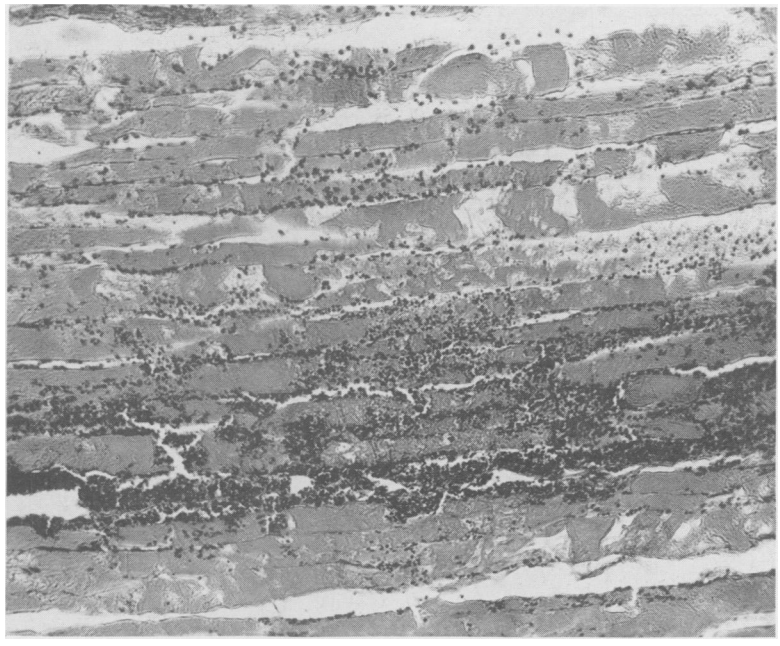

FIG. 2.—Interfibrillar infiltration of leukocytes into injured muscle tissue 48 hours after exposure to cold. Adult rabbit. H. and E. x 100.

Forty-eight hours after injury (Fig. 2), phagocytotic activity of polymorphonuclear and mononuclear cells predominated. The infiltration by the cells became so extensive as to produce interfibrillar stripes in the muscle tissue. The muscle fibers were visible as variably shaped fragments, which were encompassed by the phagocytic elements. They also lay within necrotic fibers, corroding damaged sarcoplasm, but the central portion of necrotic muscle tissue was seen to be free of leukocytes. There was a definite decrease of edema. The border areas of necrotic parts were to be replaced by proliferating connective tissue.

Muscle cell atrophy could be observed in otherwise intact histologic fields. Muscle fibers, while thinner, had more evident striations. Nuclei

were larger and stained less densely. The free interstitial spaces were relatively enlarged.

Thrombi could be observed in smaller and larger veins, while other blood vessels were dilated and well-filled with blood elements. In the necrotic parts, capillaries, though having swollen endothelial cells, showed free passage of blood (Fig. 3).

FIG. 3.—Disintegration of muscle cells 48 hours after cold injury. Absorption of necrotic fibers. Swelling of the capillary endothelium but no thrombosis. Adult rabbit. H. and E. x 175.

At 76 hours, there was increased resorption of necrotic material and increased reconstruction by proliferating connective tissue, whose fibroblasts replaced the dead muscle cells. At the same time, muscle tissue atrophy increased. Thrombi were observable in many vessels.

DISCUSSION

The course of morphologic changes observed in muscle tissue due to severe cold injury can be divided into two phases.

The first phase, characterized by extensive degeneration of muscle cells, is evident from two to six hours after injury. Exposure to cold produces the destruction of the muscle architecture. The fenestration, vacuolization and shrinkage, as well as breakage of the cells into segments fracturing into disks, represents the process of degeneration of the muscle fibers which makes the otherwise unobservable cell membranes visible. The sarcoplasma may show homogenous structure or granular distintegration. The muscle fibers lose their striation and the nuclei undergoing pyknosis and karyolysis disappear during the progress of degeneration. The acidophilic edema fluid, containing debris of nuclei and ruptured sarcolemma, distends the interstitial spaces. This process of degeneration and disintegration of muscle tissue is maximally visible from six to eight hours after injury.

The second phase is that of reconstruction subsequent to degeneration. It is effected by the infiltration of polymorphonuclear leukocytes, macrophages and later, of mononuclear cells into the damaged areas. There is a progressive activity of these elements to absorb and remove the dead muscle material. The injured tissue is eventually replaced by connective tissue

produced by proliferation of fibroblastic elements which initiates in the region of the blood vessels.

Summarizing the histologic evidence, it can be concluded that the necrosis in muscular tissue appears to be the result of the direct action of lower temperatures. The almost immediate visibility of the action of cold, and the progressiveness and continuity of events leading to tissue loss, show that the pathologic changes are initiated by exposure of the tissues to severe cold. It was already suggested by Blackwood,[1] Blackwood and Russel,[2] Greene,[9] Lewis and Love,[13] that such degenerative changes were due to this direct action of cold. Lewis,[14, 15] in his recent work noting pathologic changes in the exposed muscular tissue as early as 15 minutes after injury, confirms this point of view.

This, however, is contrary to the attitude taken by Friedman[5-7] and others,[4, 11, 12] who observed no morphologic changes in muscle two days after cold injury. They considered the necrosis to be the result of vascular occlusion by thrombi. Lewis[14] found that thrombi formation occurred only after 24 hours, and the present investigations show formation of thrombi only after 36 hours following exposure to cold. However, obvious tissue damage is to be found well before this time (two hours after exposure) which seems to negate any explanations other than that of direct action of cold.

The plugging of capillaries with red blood cells, as observed by Lange[11, 12] does not explain the severe changes in muscle cells in the early stages of frostbite, since the plugging occurs not earlier than 48 hours after injury (Johnson[10]).

The possibility of frostbite necrosis due to anoxia can be excluded by experimental results of Lewis,[14, 15] who demonstrated that muscle tissue from animals sacrificed immediately after 45 minutes of ischemia show no morphologic changes.

Boetcher[3] established a morphologic gradation of various tissues of least to most sensitivity to cold. The order of this gradation is as follows: cartilage, ligament, blood vessel, cutis, epidermis, bone, muscle, nerve, bone marrow. A similar gradation was found in our morphologic study, with the refinement that the larger blood vessels containing muscle fibers appear to be more sensitive to cold than the minute vessels. This is evident from the more distinctive vascular changes observed in vessels containing smooth muscle fibers in the wall, as noted in later stages after exposure (after 48 hours). The damage of the capillary endothelium appears to be decidedly less extensive.

This sensitivity seems to be dependent on time of exposure and temperature during exposure. By varying either of these factors, similar morphologic injuries can be produced, as can be seen in the experiments of Lewis[14, 15] and Blackwood.[1, 2] Therefore, the lower the temperature to be applied, the shorter the time of exposure must be to produce a standard injury.

SUMMARY AND CONCLUSIONS

1. Tissue changes produced by severe cold were studied in muscle tissue of rats and rabbits in various intervals after exposure, ranging from two to 76 hours.

2. Two phases in tissue changes are distinguished. The first phase of extensive degeneration is observed almost immediately after exposure to low temperatures; the second phase of reconstruction begins from about the eighth hour after exposure.

3. The formation of thrombi in the vessels was observed not earlier than 36 hours after exposure.

4. The degenerative changes in muscle tissue produced by severe cold are apparently due to direct action of low temperatures on tissue cells, as no vascular changes can be detected in the first phase.

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